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The Ferredoxin-Thioredoxin Reductase Variable Subunit Gene from *Anacystis nidulans*

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The ferredoxin-thioredoxin reductase variable subunit gene of *Anacystis nidulans* was cloned, and its nucleotide sequence was determined. A single-copy 219-bp open reading frame encoded a protein of 73 amino acid residues, with a calculated M_r of 8,400. The monocistronic transcripts were represented in a 400-base and a less abundant 300-base mRNA form.

Ferredoxin-thioredoxin reductase (FTR) is a four-iron/four-sulfur protein present in oxygenic photosynthetic organisms. It plays a central role in the ferredoxin/thioredoxin regulatory chain by converting an electron signal (photoreduced ferredoxin) to a thiol signal (reduced thioredoxin) in the regulation of enzymes by reduction of specific disulfide groups (5, 11). Native FTR isolated from a variety of sources has an approximate M_r of 30,000 and consists of an immunologically conserved similar subunit (M_r 13,000) and an immunologically variable subunit with a characteristic size in each organism studied (M_r 7,000 to 16,000) (5). While a catalytically active ferredoxin-linked disulfide has been identified as a part of the active site on the similar subunit (3, 4, 6), little is known about the variable subunit. To add to this area of knowledge, we have cloned and characterized the FTR variable subunit (FTR-V) gene from *Anacystis nidulans* 6301 (also called *Synechococcus* sp.).

Isolation of the FTR-V gene. FTR-V isolated from *A. nidulans* resembled its *Nostoc muscorum* counterpart immunologically and in molecular weight (5). The N terminus of FTR-V was accessible for protein microsequencing and yielded, with the exception of one ambiguous amino acid, a sequence of 15 amino acids (Fig. 1). On the basis of this sequence, and relying on the codon usage information available on unicellular cyanobacteria (1), we designed a 20-base 24-fold degenerate DNA probe with an estimated T_d of 58 to 66°C (Fig. 1). Under high-stringency conditions, the probe hybridized in Southern blot analysis with two bands of equal intensity, using *Anacystis* DNA cleaved with *Eco*RI and *Pst*I (Fig. 2). With the *Pst*I-digested sample, the bands identified corresponded to 2.7 and 3.8 kbp (Fig. 2). *Pst*I fragments of the hybridizing regions were extracted from the agarose gel and introduced into a pUC19 plasmid vector. Following transformation, plasmid-containing *Escherichia coli* colonies were screened by colony hybridization using the labeled oligonucleotide probe (19). Six clones, five containing the 2.7-kbp fragment and one containing the 3.8-kbp fragment, were isolated from the plasmid library.

Sequence analysis. The locations of the regions hybridizing to the synthetic oligonucleotide probe were determined by restriction site mapping. On the basis of the maps, we used a subcloning strategy that permitted DNA sequence determination from both complementary strands. The nucleotide sequence around the hybridizing region of the 3.8-kbp *Pst*I fragment contained a 219-bp open reading frame, which showed exact homology with the oligonucleotide probe (Fig. 3). Identity as the gene coding for FTR-V was based on a comparison between the amino acid sequence derived from the DNA segment and the experimentally determined N-terminal sequence of 15 amino acids shown in Fig. 1. The translation start (ATG-1) is preceded by a weak ribosome binding site (AAG-7) which is complementary to the 3′ end of the *Anacystis* 16S rRNA sequence (2, 15). The translation stop signal at the end of the gene (TAA-220) is repeated by another one (TAA-247) in the same reading frame to ensure efficient termination. The coding region is followed by a 12-bp inverted repeat (236 to 247 and 262 to 273) which appears to be a typical prokaryotic transcription terminator structure. The only other major open reading frame of the sequence proceeds from beyond position 420 toward the FTR-V gene and terminates at 276, before reaching the inverted repeat.

The 2.7-kbp *Pst*I fragment contained a 20-bp sequence that was 95 and 85% homologous to the oligonucleotide probe and the initial part of the FTR-V gene, respectively. However, the putative open reading frame of 78 bp was much shorter than expected for this gene, and there was no similarity between the determined and the derived amino acid sequences apart from that mentioned above. We con-

```
                                                                10
                                                                 *
AMINO ACID SEQUENCE  Met Asn Val Gly Asp Arg Val Arg  ?  Lys Glu Ser Val Val Val

                              10          20
                               *           *
DNA SEQUENCE         ATG AAC GTN GGN GAC AGN GT
                           T           T C

                              10          20
                               *           *
PROBE SEQUENCE       ATG AAC GTC GGC GAC CGC GT
                               G   T   T   T
                               T
```

FIG. 1. N-terminal amino acid sequence of FTR-V, corresponding DNA sequence, and sequence of the oligonucleotide probe used for isolating the FTR-V gene. FTR was purified as described previously (5), and its subunits were separated by SDS-polyacrylamide gel electrophoresis (10). Proteins were electroblotted on Immobilon membranes (14), and Edman sequence analysis was carried out with an Applied Biosystems 477A microsequencer. The oligonucleotide probe was designed on the basis of the N-terminal amino acid sequence and synthesized by using an Applied Biosystems oligonucleotide synthesizer.

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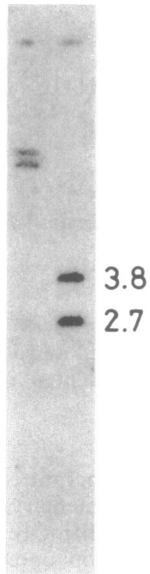

FIG. 2. Southern hybridization of total *Anacystis* DNA with the oligonucleotide probe. The probe (Fig. 1) was labeled at its 5′ end by using T4 polynucleotide kinase and [γ-^{32}P]ATP (5,000 Ci/mmol; Amersham). *Anacystis* DNA was isolated as described previously (7). Restriction fragments were separated and transferred by standard protocols (12). Hybridization was carried out as described previously (20). The left and right lanes contained 0.5 μg of *Eco*RI- and *Pst*I-cleaved DNA, respectively. Numbers indicate fragment sizes in kilobase pairs.

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                                                                    -120
           *         *         *         *         *         *
ACT CCC AAC CTA GGT GTT CCA GTC GGG CCC AGC GAT CGT TAA ACT CTT GGG CCC TGC TCC

                                                                     -60
           *         *         *         *         *         *
TCA GAA TGA TGG CTC TCT GCT AGC AAG CTC AGA GAC TAT GGG AAG ATA GAG GTT GCT TCT

                                                                       0
           *         *         *         *         *         *
AGC AGT GCT CAG GTC TCT GGG CCT CAA GCT GTG TTG TCG TCT CTG CCT CAT TAA GCT GTT

                                                                      60
           *         *         *         *         *         *
ATG AAC GTT GGC GAT CGC GTG CGA GTC AAA GAA TCA GTG GTG GTG TAT CAC CAT CCT GAT
 M   N   V   G   D   R   V   R   V   K   E   S   V   V   V   Y   H   H   P   D

                                                                     120
           *         *         *         *         *         *
CAT CGA AAT CAA GCC TTT GAC TTA AAA GAC GCG GAA GGC GAA ATT GCA GCG ATT CTA ACT
 H   R   N   Q   A   F   D   L   K   D   A   E   G   E   I   A   A   I   L   T

                                                                     180
           *         *         *         *         *         *
GAG TGG AAT GGT AAG CCC ATT AGT GCA AAC TTT CCC TAT CTC GTG AGC TTT TCC AAT AAG
 E   W   N   G   K   P   I   S   A   N   F   P   Y   L   V   S   F   S   N   K

                                                                     240
           *         *         *         *         *         *
TTT CGT GCA CAT TTA CGT GAC TTC GAG CTA GAG GTC ATC TAA ATA TGT ATT TTT GAT TGC
 F   L   A   H   L   R   D   F   E   L   E   V   I   -                  <------

                                                                     300
           *         *         *         *         *         *
CTT CAA TAA TTG ATT CAA GAA ATT GAA GCC AAT TAT CGC CAT AGC AAA CGA GCC TTC CTC
-------                     --------------->

                                                                     360
           *         *         *         *         *         *
AGC ATA AAT TTG AGT ACC GTC TCT TCA TTG GTA ATA ATG TCG GCT TCG GCA TCC ATC CCA

                                                                     420
           *         *         *         *         *         *
GGA CGG ATT TTA CAA ACC CGT TGA CCA TCA GTT AGA GAA CGA TCT TCA GCC TTG ATC GTC
```

FIG. 3. Nucleotide and derived amino acid sequences of the *A. nidulans* FTR-V gene. Numbering starts from the first translated position. Arrows indicate a putative terminator structure downstream of the coding region. Single-stranded template DNA was obtained by using the pUC118 and pUC119 vector systems (18). Sequence determination was made by the chain termination method (13), using [α-^{35}S]dATP (1,000 Ci/mmol; Amersham) and chemically modified T7 DNA polymerase (Sequenase; United States Biochemical Corp.).

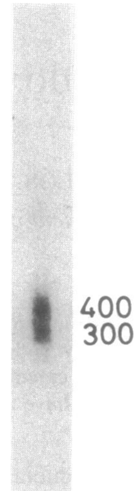

FIG. 4. Northern blot analysis of the FTR-V transcripts. *A. nidulans* was grown at 25°C in 20-liter glass bottles containing BG-11 medium, with constant aeration, in fluorescent white light (1.5×10^4 mW m^{-2}) (8). Cells were harvested from an exponentially growing culture. Total RNA was isolated as described previously (7). RNA (10 μg) was separated on a formaldehyde-containing agarose gel, blotted, and hybridized with the labeled *Pvu*I-12-to-*Apa*LI-185 fragment of the FTR-V gene. Numbers indicate RNA size in bases.

clude that the FTR-V gene found in the 3.8-kbp *Pst*I fragment is the only copy in the *A. nidulans* 6301 genome.

The FTR-V gene encodes a protein of 73 amino acid residues with a derived isoelectric point of 5.66. The calculated molecular weight (M_r 8,400) is in good agreement with the value of 7,000 for *Nostoc* (5) and *Anacystis* (data not shown) FTR-V determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The FTR-V protein is rich in aromatic amino acids (16.4% of the residues) but, significantly, seems to lack cyst(e)ine. Unlike most cyanobacterial proteins, the methionine corresponding to the initiation codon is not removed posttranslationally. A short amino acid sequence, Asn-Gly-Lys-Pro (residues 43 to 46 of FTR-V), is identical with the ferredoxin binding site of spinach ferredoxin-NADP$^+$ reductase (21). It seems possible, therefore, that this region of the *Anacystis* FTR-V protein is part of the domain interacting with ferredoxin (5, 9), especially as this same sequence is present in cyanobacterial (*Spirulina*) ferredoxin-NADP$^+$ reductase (21).

FTR-V transcript. The FTR-V gene transcript was identified in a Northern (RNA) hybridization experiment by using an internal fragment (*Pvu*I-12 to *Apa*TI-185) of the DNA coding region as a radiolabeled probe. Two transcript bands, 300 and 400 bases, were detected in a total RNA preparation from exponentially growing *A. nidulans* (Fig. 4). Both mRNA forms were short, only about 80 and 180 bases longer than the size required to accommodate the coding region. We assume, therefore, that the FTR-V gene is transcribed as a monocistronic message. The occurrence of two RNA species may be due to either (i) posttranscriptional processing or (ii) the presence of two separate transcription initiation signals in the upstream DNA sequence, TAGGTGT at −169 and TATGGG at −83, both of which resemble the *E. coli* −10 motif and seem to be within the variation range found in cyanobacteria (17). Results of Western immunoblot experiments indicated that FTR-V was not expressed from its own promoter in *E. coli*.

In summary, we have cloned and sequenced the gene encoding the variable subunit of *Anacystis* FTR. There is no similarity between the amino acid sequence derived from this gene and the terminal sequences so far available for the variable subunit (subunit A) of spinach FTR (16). A computer search also did not reveal extensive homology between FTR-V and known plant and cyanobacterial DNA sequences.

Nucleotide sequence accession number. The nucleotide sequence of the entire 849-bp *Hae*III fragment has been recorded in the EMBL data library under accession number X54196.

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